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Curtis Everett Askelson
Iowa State University

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AMINO ACID SUPPLEMENTATION OF A CORN-
SOYBEAN OIL MEAL CHICK RATION

by

Curtis Everett Askelson

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In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

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INTRODUCTION

Protein is one of the scarcest food commodities in the world. If the world's population increases at the predicted rate, protein shortages will become even more severe in the future. Even though proteins of vegetable origin are used in feeding more than two-thirds of the world's population, it has become increasingly clear that vegetable proteins are not of high biological value. Meat protein, whether beef, pork or poultry, provides the means of improving diets of the under-nourished with respect to a more favorable amino acid intake.

The conversion of feed proteins to meat proteins is far from being efficient. Poultry are one of the most efficient of our domestic animals in the conversion of feed proteins to meat protein; even in this case, only 18 percent of the protein fed can be recovered in the carcass. Future generations may have to decide whether or not meat proteins will play an increasing or decreasing role in providing adequate protein nutrition for the world's population. Because of these factors, it is of importance that today's generation attempt to further elucidate means of providing meat proteins more efficiently.

It has become accepted that feed protein(s) must be balanced in essential amino acids in order to insure a maximum growth rate in the non-ruminant animal. In most instances, diets formulated for non-ruminant animals are deficient or limiting in one or more of the essential amino acids. Three basic approaches have been taken to formulate diets so that each essential amino acid is supplied at an adequate level:

1. Increasing total protein of the diet.
2. Providing several protein sources, including both vegetable and animal proteins.
3. Supplementing the diet with crystalline amino acids.

The first approach has one serious drawback: protein wastage. The use of several protein sources in the diet results in an increased total cost of the feed. Supplementation with crystalline amino acids, with the exception of lysine and methionine, has not been feasible nor practical because of their cost and availability.

Recently it has been reported that supplementation of low protein turkey starter rations with certain essential amino acids resulted in equal performance as observed when the normal higher protein rations were fed. Similar observations have been made in laying hen rations. Although the cost of extensive use of crystalline amino acids is prohibitive at the present, if further research verifies that dietary protein may be decreased through amino acid supplementation, the future costs of these amino acids may become competitive with that of intact proteins. Thus, the more extensive use of crystalline amino acids in non-ruminant nutrition may result in a more efficient use of intact proteins in the production of meat proteins for human consumption.

The primary objective of the studies reported herein was to investigate the feasibility of crystalline amino acid supplementation of an 18 percent protein broiler chick diet, in which corn and soybean oil meal were the only sources of intact protein, in its ability to support a rapid and efficient production performance. Secondary consideration was given to the effect of protein level and dietary amino acid balance on serum protein and cholesterol concentrations.

REVIEW OF LITERATURE

Amino Acid Supplementation Studies

There have been hundreds of experimental papers published that are concerned with crystalline amino acid supplementation of various protein sources for poultry rations. Protein sources used to conduct these experiments include cottonseed meal, corn gluten meal, peanut meal, pea meal, safflower seed oil meal, sunflower seed oil meal, whole-wheat protein, meat scraps, fish meal, isolated soybean protein, casein, zein, linseed oil meal, sesame meal, soybean oil meal and various combinations of these protein sources.

The experiments conducted for and reported in this dissertation pertain to amino acid supplementation of a corn-soybean oil meal diet for growing chicks. In view of the over-all objectives set forth for these experiments, only those publications that have been involved with amino acid supplementation of diets containing soybean oil meal as the principal protein source will be considered in this section of the Review of Literature.

Prior to World War II there were numerous reports on the feeding of poultry rations containing high levels of soybean oil meal as the principal source of protein. These reports generally agreed that such rations must contain small quantities of some protein supplement other than soybean oil meal if uniformly good chick growth and feed efficiency were to be obtained. Several investigators became interested in studying what specific factors were deficient in a corn-soybean oil meal diet that resulted in poorer chick performance than that obtained when the diet contained several protein sources.

It was not until 1941 that sulfur-containing amino acids were used to supplement soybean oil meal in the diet of the chick. In this connection, Hayward and Hafner (1941) investigated cystine and methionine as supplements to raw and cooked soybeans for chicks. These workers reported that the protein of raw soybeans was greatly improved by 0.3 percent cystine and even more so by the addition of 0.3 percent DL-methionine. The protein of cooked soybeans was equally improved by supplementation of either cystine or methionine at a level of 0.3 percent of the diet.

Almquist _et al_. (1942) reported a study in which chicks were fed soybean oil meal at the 20 percent protein level and with various amino acids (methionine, cystine, lysine, tryptophan, arginine and glycine) supplemented. Their results indicated that the addition of methionine improved chick growth. The addition of lysine in combination with methionine resulted in no further improvement in chick growth. The greatest growth was observed when all six amino acids were supplemented together, but it was concluded that the majority of this increased growth could be explained by the presence of supplemental methionine.

In later reports, Grau and Almquist (1943) and Almquist and Grau (1944) described experiments with a corn-soybean oil meal diet which showed that supplemental methionine could be replaced by a combination of homocystine and choline, and that methionine could partially, but not completely, replace choline.

Bird and Mattingly (1945) obtained a significant improvement in growth of chicks when 0.2 percent DL-methionine was added to a 21 percent protein diet. The growth stimulus due to methionine supplementation slightly exceeded that obtained by supplementation with 4 percent fish meal.

Clandinin et al. (1946) reported that a practical soybean oil meal chick starter diet was improved by supplementation of methionine, and that choline and methionine could not be considered as interchangeable supplements to a practical diet.

In a study designed to investigate amino acid deficiencies of raw and overheated soybean oil meal for chicks, McGinnis and Evans (1947) found that chick growth was not improved by adding cystine to a diet containing raw soybean oil meal. The addition of methionine to this diet gave a growth response, but not at a maximum. When lysine, methionine and cystine were supplemented to soybean oil meal which had been autoclaved at 100° C. for 30 minutes, no improvement in chick growth was observed. However, the growth depression caused by autoclaving the soybean oil meal for 60 minutes was corrected by the supplementation of these three amino acids.

Gerry et al. (1948) reported experiments to study the effect of additions of methionine and/or choline chloride to a diet consisting largely of corn and soybean oil meal. When diets of 18.4, 20.0, and 21.5 percent protein were fed with additions of 0.2, 0.25 and 0.3 percent methionine or choline chloride, the lower protein level with the higher levels of either supplement resulted in improved chick growth. Although the additions of choline chloride to this simplified diet resulted in a highly significant increase in growth, the addition of methionine was more effective.

Ringrose and Potter (1952) fed a diet in which the main source of protein was soybean oil meal and studied the effects of added methionine, vitamin B_{12} and fish meal on chick growth and feed efficiency. Methionine improved chick growth when added to the basal diet, but not significantly. When methionine was added in the presence of vitamin B_{12} and fish meal, growth rate was not improved, but the feed required per unit of gain was improved to a point approaching statistical significance.

Machlin et al. (1952) reported an experiment in which chicks were fed a 22 percent protein corn-soybean oil meal diet with combinations of supplemental methionine, tryptophan and glycine. The addition of methionine alone to the basal diet resulted in chick growth equal to that observed when all three amino acids were supplemented.

Saxena and McGinnis (1952) reported that methionine did not improve the growth rate of chicks receiving 0, 2.5 and 5 percent fish meal. However, a highly significant improvement in feed efficiency was noted when methionine was added to these rations. This improvement in feed efficiency was most pronounced in groups of chicks receiving fish meal.

Matterson et al. (1953) reported that supplemental methionine in practical broiler diets significantly improved efficiency of feed utilization, whereas its effect on growth was variable. Even though added fish meal resulted in marked improvement in weight gains and feed efficiency, the presence of fish meal did not prevent supplemental methionine from improving feed efficiency. It was also reported that when chicks grown on the floor had their diets supplemented with methionine, the improvement in feed efficiency was more marked than when chicks were battery-raised.

Stephenson (1954) supplemented methionine, lysine and tryptophan singly and in various combinations to chick diets with protein levels ranging from 16 to 21 percent. Results indicated that methionine was of no value in promoting chick growth when added to a diet containing adequate protein. However, when the protein level of the diet was reduced, the addition of methionine and lysine promoted growth.

Snyder et al. (1956) reported that a practical corn-soybean oil meal diet supplemented with 0.2 percent methionine was not improved by further supplementation with gelatin. Neither arginine, glycine, nor combinations of the two amino acids, improved the growth-promoting ability of the basal diet.

An experiment on the replacement of protein of corn-soybean oil meal diets with crystalline amino acids was reported by Gordon et al. (1957). Chicks fed a 21 percent protein diet supplemented with 0.18 percent methionine hydroxy analogue and 0.2 percent glycine grew at a faster rate than did chicks fed a 25 percent protein corn-soybean oil meal diet. In another experiment the growth of chicks receiving an 18 percent protein diet, supplemented with 0.3 percent methionine hydroxy analogue and 0.6 percent glycine, approached that of chicks receiving a 24 percent protein diet.

Jimenez and Stephenson (1957) reported experiments in which chicks were fed diets supplemented with various combinations of lysine, glycine, and methionine. An improved growth rate was observed when chicks were fed diets supplemented with methionine in combination with glycine. Supplementation with lysine alone, or in combination with the other two amino acids, had no effect on rate of chick growth.

Using soybean oil meal as the sole source of protein, Snetsinger and Scott (1958) reported that, at sub-optimal dietary levels of protein, marked growth responses to methionine supplementation were noted. At higher levels of protein

(30 percent) supplemental methionine was without effect. All other essential amino acids were tested in an unsuccessful attempt to determine the second limiting amino acid of soybean oil meal.

Studies on the partial replacement of dietary protein with crystalline amino acids were reported by Middendorf and Combs (1959). Chick starter diets, composed primarily of corn and soybean oil meal, were diluted with a starch-corn oil-mineral-vitamin mixture. As the calorie-protein ratio was widened to 120:1, each level of dilution decreased growth rate and feed efficiency. Supplementation with all essential amino acids failed to return chick growth to normal. The diets were also not improved by further supplementation with alanine, proline and aspartic acid. When amino acid requirements were converted to grams of amino acid per therm of productive energy, assuming the energy requirement was 900 Calories per pound of feed, amino acid mixtures following the proportions of these requirements were found to be more effective than those designed to return each amino acid to 100 percent of its requirement.

Effect of Dietary Protein on Serum Proteins

Because of the limited amount of literature available pertaining to the effect of dietary protein on the blood proteins in the avian species, a selection of the more pertinent literature of research conducted with other non-ruminant animals will also be considered in this section.

Weimer *et al*. (1959) demonstrated that feeding a protein-free diet for one week to rats resulted in highly significant decreases in total serum protein, albumin and beta globulin concentrations. The greatest changes were noted in the albumin fractions, which resulted in marked changes in albumin/globulin ratios.

The effect of increasing dietary protein from 0 to 14 percent on the turnover rate of serum proteins was reported by Jeffay and Winzler (1958). These workers observed that the turnover rate of serum albumin was increased with increasing levels of protein, while the turnover rate of the globulins remained essentially unchanged.

Friend *et al*. (1961) fed a limited number of young pigs diets varying in protein and vitamin A. The blood serum of pigs receiving the low-protein diets had lower serum albumin and serum vitamin A concentrations than did pigs receiving the higher protein diets.

Allison (1955) reported data obtained from rats fed a protein-free diet, diets containing 12 and 18 percent casein, and a 12 percent casein diet supplemented with methionine. Rats receiving the protein-free diet had lower serum protein concentrations than did rats receiving the other experimental treatments. Serum protein concentrations of rats fed the 18 percent casein and 12 percent casein plus methionine diets were greater than those of rats fed the 12 percent casein diet. These results may indicate that amino acid balance is also instrumental in producing significant changes in the blood protein concentrations. It was further observed that the albumins were more labile than the globulins, with a greater percentage change taking place in the albumin fraction than in the globulin fractions.

Serum samples obtained from 6-week-old chicks fed a normal practical diet and the same diet supplemented with 0.1 percent free gossypol were analyzed for serum proteins by Nairn et al. (1961). Lower total serum protein and serum albumin concentrations were noted when chicks were fed the gossypol-supplemented diet. In a second experiment, chicks were fed a purified-type diet at levels of 17, 21 and 42 percent protein, with and without added gossypol. Results of this experiment showed that as protein level was increased there was a corresponding increase in total serum protein, while the albumin/globulin ratio decreased as the protein level of the diet increased. When gossypol was added, an irregular trend in the serum protein fractions was observed.

Leveille et al. (1960) observed that chicks fed a low-protein diet exhibited lower total serum protein and serum albumin concentrations than chicks fed a high-protein diet. The decreased serum albumin concentrations were accompanied by a lower albumin/globulin ratio.

In a subsequent experiment, Leveille and Sauberlich (1961) fed male chicks diets containing 10, 15, 20 and 25 percent protein for a three week period. The protein level was increased by the substitution of sesame oil meal in the diet at the expense of sucrose. The protein requirement for maximum growth was found to be between the two highest protein levels fed and was calculated as 20.5 percent of the diet. Total serum protein increased from 2.33 grams per 100 ml. at the 10 percent dietary protein level to 3.06 grams per 100 ml. at the 25 percent protein level. Changes in serum concentrations were attributed to variations in the albumin concentration, the concentration of globulins remaining constant at all protein levels fed.

Effect of Dietary Protein on Serum Cholesterol

Leveille and Fisher (1958) studied the effects of adding four fat sources at three graded levels to high- and low-protein diets (25 and 8 percent protein) on serum cholesterol concentrations in growing chicks. On the low-protein diet, a marked hypercholesterolemia was observed, which was not affected by type or level of fat supplemented. On the high-protein diet, serum cholesterol concentrations were essentially normal regardless of source and level of fat.

An inverse linear relationship was found to exist between serum cholesterol concentrations and absolute intake of protein in an experiment reported by Kokatnur *et al*. (1958). In these studies 12 to 18 month old birds were fed diets of 7.5, 15 and 30 percent protein at two levels of corn oil. The largest increase in serum cholesterol was noted in birds which had consumed the least amount of protein. It was further observed that serum cholesterol concentrations decreased most rapidly in hypercholesterolemic chicks when diets adequate or high in protein were fed.

Dietary deficiencies of the amino acids arginine, lysine, methionine and tryptophan at different levels of protein intake, as related to growth and serum cholesterol in young chicks, were reported by Johnson *et al*. (1958). It was shown that at sub-optimal protein intake a hypercholesterolemia resulted which could be modified by supplementing the different proteins with amino acids. At optimal or super-normal protein intake arginine supplementation exerted a cholesterol-lowering effect, which was not noted when the other three amino acids were supplemented.

Nishida *et al*. (1958) reported that dietary protein tended to depress the atherogenic effect of dietary cholesterol and fat in chicks. A significantly elevated serum cholesterol concentration was observed when a 15 percent protein diet, deficient in methionine, was fed.

Pick *et al*. (1959) found that a high protein intake suppressed hypercholesterolemia and atherogenesis in young cockerels which were receiving a high-cholesterol, high-fat diet. It was reported that this trend was observed with various protein sources, and was not altered by source of cholesterol or type of fat.

An experiment in which male chicks were fed diets containing 20 to 26 percent protein and 0 to 8 percent fat was reported by March and Biely (1959). Birds fed the highest protein diet demonstrated faster growth rate and lower serum cholesterol concentration than birds fed the lower protein diet. Chicks receiving the low-protein and added-fat diet had elevated serum cholesterol concentrations as compared to chicks fed the high-protein plus added-fat diet.

Further experiments by Nishida et al. (1960) again demonstrated that chicks fed a sub-optimal level of protein had higher serum cholesterol concentrations than did chicks fed a 30 percent protein diet. When 1 percent cholesterol was added to each diet, the greatest increase in both serum and liver cholesterol was observed in birds fed the 15 percent protein diet. It was also noted that a low dietary protein level tended to increase initial incorporation of acetate-1-C^{14} into liver cholesterol. This was appreciably increased when 1 percent cholesterol was added to the low-protein diet.

Marion et al. (1961) found that serum cholesterol values were not influenced by dietary protein in the absence of added fat. Increasing dietary protein in the presence of fat, however, resulted in lower serum cholesterol concentrations in the chick.

Kokatnur and Kummerow (1961) tested six essential amino acids for their effect on serum cholesterol in diets which were either deficient, or in excess, in one of the amino acids. Under these conditions, arginine, lysine and leucine had an influence on serum cholesterol as well as on chick growth. Toxic levels of lysine and histidine elevated serum cholesterol and depressed growth. The toxic effects of lysine could be partially overcome by supplementing the diet with a mixture of glycine and arginine, or glycine and methionine. It was also observed that increasing dietary nitrogen intake resulted in a progressive decrease in serum cholesterol concentrations.

Leveille _et al_. (1962) studied the influence of additions of four essential amino acids to 10- and 25-percent protein diets on serum cholesterol in the growing chick. Their data demonstrated a hypocholesterolemic effect for methionine, unrelated to growth, in chicks fed diets marginal in this amino acid. No specific hypocholesterolemic effect was evident for lysine or arginine, even though lysine supplementation tended to depress serum cholesterol concentrations and stimulated growth rate in diets deficient in this amino acid.

EXPERIMENTAL PROCEDURE

Methods and Materials

General management

Vent-sexed Ames In-Cross male chicks hatched at the Iowa State University Poultry Farm were used to conduct the four-week feeding experiments reported herein. Day-old chicks were placed in five-deck battery brooders equipped with wire floors and thermostatically controlled electric heating elements of the back-warming type. The temperature under the hovers was initially 95° F. and was adjusted to the comfort of the chicks throughout the experiment. The heights of the hovers were also adjusted during the experimental period. The battery brooders were located in a room providing adequate heat, light and ventilation.

Chicks had access to feed and water *ad libitum*. Daily observations were made to insure that adequate feed and water were available to the experimental birds. Experimental rations were mixed in quantities to provide for 20 pounds of feed for each experimental pen, and stored in metal cans with covers.

Records and statistical methods

Individual chick weights and pen feed consumption data were recorded at two and four weeks of the experimental period. Blood samples for serum protein and cholesterol determinations were collected at the four week period in two of the experiments reported. Analyses of variance of chick data collected were made according to methods described by Snedecor (1956).

Collection of blood samples

In experiments four and six, blood samples were collected by means of cardiac puncture at the termination of the feeding period. A single pooled blood sample was collected for each experimental pen by removing approximately 1 1/2 ml. of blood from each individual chick within a pen. The pooled blood samples were then centrifuged to obtain the serum portion for subsequent chemical analyses.

Serum protein determination

Serum proteins were determined by the biuret reaction method described by Gornall *et al*. (1949). The procedure used was as follows:

1. 0.5 ml. of serum was transferred from a pipette to a clean centrifuge tube.
2. 9.5 ml. of a 22.6 percent sodium sulfate solution were delivered into the tube containing the serum by means of a buret.
3. The centrifuge tube was stoppered, and the contents

mixed by inversion.

4. Immediately following the mixing process, 2.0 ml. of the mixture were transferred by pipette to a clean test tube. This solution represented that portion giving the total serum protein concentration.
5. To the remaining 8 ml. of the serum-sulfate mixture, 3 ml. of ethyl ether were added by means of a buret. The resulting solution was mixed by inversion for 30 seconds, then centrifuged for 10 minutes.
6. After centrifugation, 2.0 ml. of the aqueous phase were transferred to another test tube. This solution represented that portion giving the serum albumin concentration.
7. Into each of the resulting test tubes, 8.0 ml. of biuret reagent were added by means of an automatic pipetting syringe. Samples were allowed to stand at room temperature for 30 minutes before optical density readings were obtained on a DU spectrophotometer at a wavelength of 540 millimicrons.
8. Concentrations of total serum protein and serum albumin were obtained by reference to a calibration curve, which was obtained by reacting known concentrations of human serum albumin with the biuret reagent. The serum globulin concentration was obtained by subtracting the serum albumin concentra-

tion from the total serum protein concentration for each serum sample.

10. Duplicate determinations were performed on each serum sample collected from an experimental pen.

The biuret reagent was prepared by dissolving 1.5 grams cupric sulfate ($CuSO_4 \cdot 5H_2O$) and 6.0 grams of sodium potassium tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$) in 500 ml. of distilled water. With constant swirling, 300 ml. of a 10 percent sodium hydroxide solution were added to the above mixture. A final volume of 1000 ml. of biuret reagent was obtained by the addition of distilled water.

<u>Serum cholesterol determination</u>

A modification of the method described by Zlatkis *et al.* (1953) was used for the determination of serum cholesterol. The color reagent of Rosenthal *et al.* (1957) was used in lieu of that reagent described in the former publication. An iron stock solution was prepared by dissolving 2.5 grams of ferric chloride ($FeCl_3 \cdot 6H_2O$) in 100 ml. of 85 percent phosphoric acid. Of this stock solution, 8.0 ml. were then diluted to 100 ml. with concentrated sulfuric acid to give the final color reagent. The chemical procedure used for the determination of serum cholesterol was as follows:

1. 0.1 ml. of serum was transferred from a micropipette to a clean test tube.

2. 5.0 ml. of glacial acetic acid were added to the serum from a buret.
3. Immediately following the addition of acetic acid, 4.0 ml. of the color reagent were added from a buret to the above solution.
4. The contents of each tube were thoroughly mixed and allowed to cool to room temperature.
5. Optical density readings of the solutions were obtained using a DU spectrophotometer at a wavelength setting of 560 millimicrons.
6. Serum cholesterol concentrations were calculated from a standard curve, which was recalibrated with each set of serum cholesterol analyses.
7. Duplicate determinations were performed on each serum sample collected from an experimental pen.

EXPERIMENTAL RESULTS

Experiment One

Purpose

This experiment was designed to observe the effects of crystalline amino acid supplementation of an 18 percent protein diet, which contained corn and soybean oil meal as the only sources of intact protein, on chick growth and feed efficiency.

Procedure

The experiment consisted of three dietary treatments, each triplicated, giving a total of nine experimental pens. Ninety day-old chicks were individually weighed, grouped into lots of ten chicks and assigned a battery brooder in accordance with a completely randomized design.

The composition of experimental rations is given in Table 1. The 18 percent protein basal diet was formulated so that crystalline amino acids would be supplemented in place of monosodium glutamate on an equal weight basis. It was assumed that all crystalline amino acids, including monosodium glutamate, contributed 100 percent crude protein. Thus, the two lower protein diets used in this experiment were considered isoproteinous even though they were not isonitrogenous.

Another feature of the 18 percent protein basal diet was

Table 1. Composition of diets used in experiment one

Ingredient	18% Basal	18% Basal + a.a.	22% Control
Ground yellow corn	59.2	59.20	53.5
Soybean oil meal, 50% protein	21.0	21.00	34.4
Soybean oil	9.0	9.00	7.5
Cellulose, Alphacel	4.0	4.00	--
Dicalcium phosphate	2.0	2.00	2.0
Ground oystershell	1.0	1.00	1.0
Salt mix[a]	0.6	0.60	0.6
Vitamin and antibiotic mix[b]	1.0	1.00	1.0
Monosodium glutamate	2.2	0.03	--
L-lysine HCL	--	0.31	--
DL-methionine	--	0.27	--
Glycine	--	0.80	--
L-arginine HCL	--	0.14	--
DL-threonine	--	0.21	--
DL-valine	--	0.29	--
DL-histidine HCL	--	0.06	--
DL-tryptophan	--	0.05	--
DL-isoleucine	--	0.04	--
Calculated analysis[c]			
Protein, %	18.0	18.00	22.0
Productive energy, Calories per pound	1105	1105	1102

[a]Salt mix is shown in Appendix, Table 18.

[b]Vitamin and antibiotic mix is shown in Appendix, Table 17.

[c]Values used to arrive at this analysis are given in Appendix, Table 16.

the inclusion of 4 percent cellulose. This formulation offers the capability of increasing total crude protein by additional supplementation of monosodium glutamate in place of cellulose in future experiments. These two features afford the flexibility of changing the amino acid balance and/or total crude protein percentage of this diet without altering the levels of corn and soybean oil meal.

Amino acid levels reported by Dean and Scott (1962) were used as the criteria for supplementation of crystalline amino acids to the 18 percent protein basal diet. These workers used both purified and semi-purified diets in the establishment of an amino acid standard which resulted in a maximum growth response in young chicks receiving an 18 percent protein diet (N x 6.25). The essential amino acid levels reported by these workers and the calculated amino acid composition of the 18 percent protein basal diet used in this experiment are shown in Table 2. For comparative purposes, the essential amino acid requirements recommended by the National Research Council for starting chicks receiving a 20 percent protein diet are also shown in Table 2.

With the exception of phenylalanine and leucine, it was necessary to supplement the basal diet with varying quantities of all essential amino acids in order to achieve the individual amino acid levels reported by the Illinois workers. Since crystalline cystine was not used as a supplemental

Table 2. Comparison of amino acids in basal diet versus levels reported by Dean and Scott (1962) and the National Research Council

Amino acid	18% Basal[a]	Dean and Scott (1962)	NRC[b]
Lysine	0.81[c]	1.12[c]	0.90[c]
Methionine	0.29	0.45	0.45
Cystine	0.24	0.35	0.35
Glycine	0.80	1.60	1.00
Arginine	0.96	1.10	1.20
Threonine	0.64	0.85	0.60
Valine	0.75	1.04	0.80
Histidine	0.44	0.50	0.15
Tryptophan	0.18	0.23	0.20
Isoleucine	0.76	0.80	0.60
Leucine	1.43	1.20	1.40
Phenylalanine	0.80	0.68	0.90

[a]Values used to calculate amino acid levels in this diet are given in Appendix, Table 16.

[b]Nutrient Requirements for Poultry, National Academy of Sciences, National Research Council, 1960.

[c]Expressed as percent of total diet.

ingredient, DL-methionine was added to result in a combined methionine and cystine level of 0.8 percent of the diet.

Results and discussion

Chick growth and feed efficiency data for this experiment are given in Table 3. At the end of two weeks, chicks receiving the amino acid-supplemented diet showed an 8.1 percent faster growth rate and an 11.1 percent improvement in feed

Table 3. Chick weight and feed efficiency data for experiment one

Dietary treatment	Chick weight (grams) Rep. 1	Rep. 2	Rep. 3	Av.	Feed efficiency[a] Rep. 1	Rep. 2	Rep. 3	Av.
<u>2 weeks</u>								
18% Basal	182.8[b]	175.6[b]	177.4[b]	178.6	1.63	1.68	1.65	1.65
18% Basal + a.a.	194.5	185.0	197.6	192.4	1.48	1.48	1.47	1.48
22% Control	207.8	199.0	211.6	206.1	1.40	1.44	1.36	1.40
<u>4 weeks</u>								
18% Basal	456.0	432.3	449.5	445.9	1.84	1.88	1.86	1.86
18% Basal + a.a.	485.4	475.1	496.8	485.8	1.68	1.64	1.64	1.65
22% Control	530.0	522.9	541.8	531.6	1.63	1.71	1.63	1.66

[a]Grams of feed per gram of gain.

[b]Average weight of 10 chicks per experimental pen.

efficiency as compared to chicks receiving the unsupplemented 18 percent protein diet. Analyses of variance (Appendix, Table 19) indicated that this improved growth was statistically significant at P = .05 or less, while the improved feed efficiency was statistically significant at P = .01 or less. The magnitude of response to supplemental amino acids (8.9 percent improved growth and 12.7 percent improved feed efficiency) was not appreciably altered at the end of four weeks. Both of these differences were found to be statistically significant at P = .01 or less. These results indicate that it was possible to improve chick growth and feed efficiency by dietary supplementation of amino acids to the level as reported by Dean and Scott (1962).

The 22 percent protein corn-soybean oil meal diet was included as an experimental treatment to observe whether or not chick growth obtained with the amino acid-supplemented 18 percent protein diet was comparable to that of chicks fed a practical, high-energy 22 percent protein diet. Chicks fed the higher protein diet were 7.1 and 9.4 percent heavier in body weight at two and four weeks of age, respectively, than were chicks fed the amino acid-supplemented 18 percent protein diet. In contrast to the differences in body weight, the feed conversions for chicks fed these two diets were similar, especially at the end of four weeks. Although the method selected for statistical analyses of these data did not permit an orthogonal comparison between these two experimental treatments, it is felt that the data is self-explanatory without the aid of this statistical comparison.

The failure to observe a maximum growth rate with the 18 percent protein diet, even after amino acids were supplemented at the levels indicated, may be the result of several factors. These results could suggest that even though the essential amino acid requirements were fulfilled, total nitrogen intake may have been a limiting factor in failure to support a maximum growth rate. However, it could be entirely possible that amino acid supplementation still did not provide adequate amounts of one or more of the essential amino acids. Although growth rate and feed efficiency were improved by amino acid supplementation, consideration must also be given to the possibility that an amino acid imbalance and/or toxicity was induced by supplementation of one or more of the crystalline amino acids, thereby offsetting or masking the full effect of the other amino acids supplemented.

Experiment Two

Purpose

Having observed a significant improvement in weight gain and feed efficiency by supplementation of nine essential amino acids to the 18 percent protein diet in experiment one, this study was designed to observe which of the amino acids supplemented to this diet were responsible for the improvement in chick growth and feed utilization.

Procedure

Because of battery brooder space limitations, it was necessary to sub-divide this experiment into three separate feeding trials. With the exception of experimental diets, the procedures followed in conducting these feeding trials were similar. Each feeding trial consisted of eight dietary treatments, each duplicated, giving a total of sixteen pens. One hundred and sixty day-old chicks for each feeding trial were individually weighed, grouped into lots of ten chicks and assigned a battery brooder in accordance to a 2 x 2 x 2 completely randomized factorial design.

The composition of the experimental diets used to conduct these feeding trials was the same as the 18 percent protein, amino acid-supplemented diet given in Table 1, with the following modifications:

1. In trial one, the basal diet included all supplemental amino acids with the exception of lysine, methionine and glycine, which were replaced by monosodium glutamate on an equal weight basis. The remaining seven experimental treatments were obtained by supplementation of lysine, methionine and glycine individually and in all possible combinations. As

in experiment one, amino acid supplementation was accomplished at the expense of monosodium glutamate.

2. A similar procedure as outlined above was used in the formulation of diets used in the remaining two feeding trials of this experiment. The basal diet in trial two included all supplemental amino acids except arginine, threonine and valine, whereas in trial three, all amino acids were added to the basal diet except histidine, isoleucine and tryptophan. In each of the respective trials, these amino acids were then supplemented individually and in all possible combinations to fulfill the requirements for a 2 x 2 x 2 factorial design.

Results and discussion

Chick weight and feed efficiency data for trial one of experiment two are shown in Table 4, and the analyses of variance of these data are given in Appendix, Table 20.

At two weeks, the only statistically significant (P = .05 or less) response in chick weight was observed when the basal diet was supplemented with lysine, methionine and glycine in combination. Chicks receiving this diet were 11.3 percent heavier in body weight than were chicks receiving the basal diet, which did not include supplemental amounts of these three amino acids. No significant response in body weight was

Table 4. Chick weight and feed efficiency data for trial one of experiment two

Dietary treatment	Chick weight (grams) Rep. 1	Chick weight (grams) Rep. 2	Chick weight (grams) Av.	Feed efficiency[a] Rep. 1	Feed efficiency[a] Rep. 2	Feed efficiency[a] Av.
<u>2 weeks</u>						
Basal	174.1[b]	182.4[b]	178.2	1.59	1.60	1.60
+ lysine (L)	179.9	185.3	182.1	1.54	1.54	1.54
+ methionine (M)	181.9	187.7	184.3	1.45	1.61	1.53
+ glycine (G)	186.9	179.1	182.6	1.53	1.61	1.57
+ L + M	176.7	187.6	182.2	1.54	1.45	1.50
+ L + G	178.9	186.8	182.8	1.42	1.43	1.42
+ M + G	175.8	173.2	174.5	1.57	1.67	1.62
+ L + M + G	188.3	208.3	198.3	1.70	1.42	1.56
<u>4 weeks</u>						
Basal	455.4	479.6	467.5	1.86	1.85	1.86
+ L	464.1	473.8	469.0	1.91	1.85	1.88
+ M	478.9	483.1	481.0	1.83	1.86	1.84
+ G	480.6	468.0	474.3	1.86	1.84	1.85
+ L + M	490.3	482.6	486.4	1.71	1.67	1.69
+ L + G	487.4	468.5	478.0	1.75	1.80	1.78
+ M + G	457.3	466.7	462.0	1.98	1.82	1.90
+ L + M + G	504.4	525.3	514.9	1.84	1.70	1.77

[a]Grams of feed per gram of gain.

[b]Average weight of 10 chicks per experimental pen.

observed when these amino acids were supplemented singly and in two-way combinations. These results indicate that all three of the above mentioned amino acids must be supplemented to the basal diet in order to achieve a maximal growth response up to two weeks of chick age.

Analysis of the four week body weight data revealed additional information as to the effect of supplementation of these three amino acids in that statistically significant main effects and two-way interactions were found to be present. In addition to statistically significant (P = .05 or less) main effects due to supplemental lysine and methionine, a significant lysine (L) x methionine (M) interaction was also revealed. The response in growth to supplemental lysine in the presence of supplemental methionine was found to be statistically different (P = .05 or less) from that observed when supplemental methionine was not added. Supplementing the diet with lysine alone caused an improved growth rate of less than 1 percent. When methionine was supplemented alone, an improved growth rate of 2.9 percent was observed. In contrast, chicks fed diets supplemented with lysine and methionine in combination were 4.0 and 10.1 percent heavier in body weight than were chicks fed the basal diet.

Although the main effect on chick growth due to glycine supplementation was non-significant, there was a statistically significant (P = .05 or less) lysine (L) x glycine (G) interaction. The supplementation of glycine in the absence of supplemental lysine did not result in an improvement in chick growth. However, when glycine was added in the presence of supplemental lysine, chick growth was improved. The nature of the L x M and L x G interactions would lead one to suspect that a significant L x M x G interaction, as was observed at two weeks, would be present. However, statistical analysis failed to verify that this three-way interaction was significant at the probability of .05, although it did approach significance at this level.

At two weeks of chick age, supplementation of lysine, methionine and glycine, either singly or in combination, had no significant effect on feed efficiency. At four weeks, however, significant differences in feed efficiency were observed among the various dietary treatments. Similar to body weight data, statistically significant (P = .05 or less) lysine and L x M interaction effects upon feed efficiency were revealed. In addition, the L x M x G interaction was also found to be statistically significant at the same level of probability. The nature of these interactions suggests that a maximal response in feed efficiency was obtained by supplementation of all three amino acids in combination.

The response in feed efficiency to supplemental glycine in the presence of supplemental methionine was found to be statistically different (P = .05 or less) from that observed when glycine was added in the absence of supplemental methionine. Feed efficiency of chicks fed diets containing additions of glycine or methionine alone did not differ from that of chicks fed the basal diet. However, when these two amino acids were supplemented in combination, there was a definite tendency to increase the feed required per unit of chick gain. At the same time, it will be noted that the lowest body weights observed in this trial were obtained with chicks that were fed supplemental methionine and glycine in combination. Thus, the data on body weights and feed efficiency for chicks fed supplemental methionine and glycine in combination are similar.

Chick weight and feed efficiency data for trial two of experiment two are given in Table 5, and the analyses of variance of these data are given in Appendix, Table 21.

The results of this trial indicate that no significant improvements in chick weight and/or feed efficiency were obtained at two or four weeks of age when arginine, threonine and valine were supplemented, either singly or in various combinations, to the basal diet. In fact, the body weights of chicks receiving diets supplemented with these three amino acids were lower, but not significantly lower, than that observed for chicks receiving the basal diet.

There was, however, a statistically significant (P = .05 or less) threonine x valine interaction at four weeks of chick age. Supplementation of threonine or valine individually resulted in weight gains of only 92.0 and 96.5 percent,

Table 5. Chick weight and feed efficiency data for trial two of experiment two

Dietary treatment	Chick weight (grams)			Feed efficiency[a]		
	Rep. 1	Rep. 2	Av.	Rep. 1	Rep. 2	Av.
2 weeks						
Basal	201.1[b]	189.4[b]	195.2	1.44	1.52	1.48
+ arginine (A)	194.2	170.0	182.6	1.46	1.49	1.48
+ threonine (T)	183.7	182.9	183.3	1.38	1.41	1.39
+ valine (V)	184.1	186.4	185.2	1.43	1.41	1.42
+ A + T	192.7	172.0	184.2	1.45	1.66	1.56
+ A + V	200.3	183.9	192.1	1.41	1.46	1.44
+ T + V	198.4	182.9	190.6	1.52	1.42	1.47
+ A + T + V	188.1	192.9	190.5	1.67	1.35	1.51
4 weeks						
Basal	514.9	496.4	505.7	1.74	1.68	1.71
+ A	501.8	496.5	499.2	1.76	1.71	1.74
+ T	474.1	456.5	465.3	1.67	1.71	1.69
+ V	479.2	497.3	488.2	1.69	1.70	1.70
+ A + T	488.8	471.8	480.3	1.72	1.73	1.72
+ A + V	516.0	478.0	497.0	1.70	1.79	1.74
+ T + V	499.1	484.2	491.6	1.72	1.88	1.80
+ A + T + V	508.0	500.7	504.4	1.71	1.61	1.66

[a]Grams of feed per gram of gain.

[b]Average weight of 10 chicks per experimental pen.

respectively, of that observed for chicks receiving the basal diet. When these two amino acids were supplemented in combination, chick growth was increased up to 97.2 percent of that observed for the basal diet. It would appear that when threonine was supplemented individually an amino acid imbalance occurred, which resulted in a tendency to depress chick growth.

Chick weight and feed efficiency data for trial three of experiment two are given in Table 6, and the analyses of variance of these data are given in Appendix, Table 22.

Supplementation of the basal diet with histidine, tryptophan and isoleucine individually or in various combinations did not improve the rate of chick weight gains over that observed for the basal diet. The only statistically significant ($P = .05$ or less) effect due to amino acid supplementation in this trial was the increased feed requirement per unit of gain of chicks that received supplemental tryptophan. Histidine and isoleucine supplementation had no significant effect on feed utilization.

The results of these three feeding trials indicate that the basal diet was deficient in lysine, methionine and glycine in its ability to support maximal chick growth at this particular protein level. Of these three amino acids, it appears that methionine was the first limiting amino acid, followed by lysine, then glycine. A maximal response in chick weight

Table 6. Chick weight and feed efficiency data for trial three of experiment two

Dietary treatment	Chick weight (grams)			Feed efficiency[a]		
	Rep. 1	Rep. 2	Av.	Rep. 1	Rep. 2	Av.
2 weeks						
Basal	204.6[b]	212.2[b]	208.4	1.41	1.43	1.42
+ histidine (H)	209.0	212.7	210.8	1.48	1.43	1.46
+ tryptophan (T)	212.8	205.5	209.2	1.42	1.43	1.42
+ isoleucine (I)	219.6	208.6	214.1	1.39	1.46	1.42
+ H + T	203.0	210.9	207.0	1.45	1.41	1.43
+ H + I	216.7	215.4	216.0	1.44	1.40	1.42
+ T + I	206.9	199.0	203.0	1.44	1.54	1.49
+ H + T + I	211.3	218.1	214.7	1.46	1.46	1.46
4 weeks						
Basal	526.8	536.9	531.8	1.59	1.64	1.62
+ H	531.9	517.6	524.8	1.70	1.74	1.72
+ T	531.2	478.6	504.9	1.64	1.76	1.70
+ I	550.8	507.2	529.0	1.64	1.67	1.66
+ H + T	502.0	527.3	514.6	1.71	1.68	1.70
+ H + I	516.1	520.8	518.4	1.76	1.66	1.71
+ T + I	511.1	497.7	504.4	1.79	1.74	1.76
+ H + T + I	508.2	535.2	521.7	1.73	1.79	1.76

[a]Grams of feed per gram of gain.

[b]Average weight of 10 chicks per experimental pen.

gain and feed efficiency was not obtained unless the basal diet was supplemented with these amino acids in combination. Further supplementation of the basal diet with arginine, threonine, valine, histidine, tryptophan and isoleucine did not improve chick growth or feed efficiency.

Experiment Three

Purpose

This experiment was designed as a positive check to insure that the basal diet used in experiment one required only the supplementation of lysine, methionine and glycine to result in maximum chick growth and feed utilization.

Procedure

The experiment consisted of three dietary treatments, each triplicated, giving a total of nine experimental pens. Ninety day-old chicks were individually weighed, grouped into lots of ten chicks and assigned a battery brooder in accordance with a randomized block design.

The experimental treatments were as follows:

1. 18 percent protein basal.
2. 18 percent protein basal plus three amino acids.
3. 18 percent protein basal plus nine amino acids.

The composition of diets 1 and 3 is given in Table 1. Diet 2 was a modification of the basal diet in that only lysine, methionine and glycine were supplemented in place of monosodium glutamate on an equal weight basis.

Results and discussion

Chick weight and feed efficiency data for experiment three are given in Table 7, and the analyses of variance of these data are given in Appendix, Table 23.

At two weeks of age, body weights of chicks whose diets had been supplemented with either three or nine amino acids were 14.0 and 11.2 percent heavier, respectively, than chicks fed the basal diet. These differences were found to be statistically significant at P = .01 or less. As would be expected, chicks receiving the amino acid-supplemented diets demonstrated a significantly (P = .01 or less) improved feed efficiency when compared with chicks receiving the basal diet.

The magnitude of response in chick weight and feed efficiency due to amino acid supplementation was not altered at the end of four weeks. Again, chicks fed the amino acid-supplemented diets showed significantly (P = .01 or less) improved body weights and feed efficiency over that observed for chicks fed the basal diet.

At both two and four weeks of age, there were no significant differences in body weight and feed efficiency between those chicks receiving diets supplemented with lysine, methionine and glycine, and those chicks receiving diets supple-

Table 7. Chick weight and feed efficiency data for experiment three

Dietary treatment	Chick weight (grams) Rep. 1	Rep. 2	Rep. 3	Av.	Feed efficiency[a] Rep. 1	Rep. 2	Rep. 3	Av.
2 weeks								
Basal	189.3[b]	196.8[b]	195.2[b]	193.8	1.63	1.62	1.68	1.64
+ 3 a.a.	221.7	218.2	222.8	220.9	1.47	1.42	1.44	1.44
+ 9 a.a.	202.1	226.4	217.9	215.5	1.43	1.43	1.44	1.43
4 weeks								
Basal	468.0	484.6	470.6	474.4	1.87	1.86	2.06	1.93
+ 3 a.a.	553.7	516.3	549.1	539.7	1.70	1.74	1.66	1.70
+ 9 a.a.	534.3	560.8	533.6	542.9	1.60	1.63	1.66	1.63

[a]Grams of feed per gram of gain.

[b]Average weight of 10 chicks per experimental pen.

mented with all nine amino acids used in previous experiments. The results of this experiment support the observations made in experiment two, i.e., that a maximal response in chick growth can be obtained when the basal diet is supplemented with lysine, methionine and glycine in combination, and that further supplementation of this diet with arginine, threonine, valine, histidine, tryptophan and isoleucine does not significantly improve chick weight and feed efficiency.

Experiment Four

Purpose

This experiment was designed to observe the effects of increasing total crude protein of the basal diet used in previous experiments, with and without essential amino acid supplementation, on chick growth, feed efficiency, serum protein and serum cholesterol concentrations.

Procedure

The experiment consisted of nine dietary treatments, each duplicated, giving a total of eighteen experimental pens. One hundred and eighty day-old chicks were individually weighed, grouped into lots of ten chicks and assigned a battery brooder in accordance with a completely randomized design.

Procedures used for the collection of blood samples and subsequent analyses are outlined in the Experimental Procedure section.

The composition of experimental rations is given in Table 8. There were basically three types of experimental diets used in this investigation. First, there were three "basal" diets consisting of 18, 20 and 22 percent protein. The essential amino acid composition of these diets was identical, since the level of crude protein was increased by replacing cellulose on an equal weight basis with monosodium glutamate.

The second type of diet was formulated by the supplementation of nine essential amino acids to each of the three basal diets discussed above. Again, the essential amino acid composition of these three diets was identical.

In contrast to this general type of formulation, the crude protein level of the three "control" diets used in this experiment was increased by replacing corn with soybean oil meal. Thus, the essential amino acid compositions of these diets were not constant across protein levels. It was also necessary to increase the level of soybean oil as the level of protein was adjusted upward, in order to maintain a constant energy level.

On a calculated basis, the energy contents of all nine experimental diets were within $\pm$ 3 productive Calories per pound of feed when compared with one another.

Table 8. Composition of diets used in experiment four

Ingredient	Basal 18%	Basal 20%	Basal 22%	Basal + a.a. 18%	Basal + a.a. 20%	Basal + a.a. 22%	Control 18%	Control 20%	Control 22%
	Percentage composition								
Ground yellow corn	59.2	59.2	59.2	59.20	59.20	59.20	65.7	59.6	53.5
Soybean oil meal, 50% protein	21.0	21.0	21.0	21.00	21.00	21.00	24.2	29.3	34.4
Soybean oil	9.0	9.0	9.0	9.00	9.00	9.00	5.5	6.5	7.5
Cellulose, alphacel	4.0	2.0	--	4.00	2.00	--	--	--	--
Dicalcium phosphate	2.0	2.0	2.0	2.00	2.00	2.00	2.0	2.0	2.0
Ground oystershell	1.0	1.0	1.0	1.00	1.00	1.00	1.0	1.0	1.0
Salt mix[a]	0.6	0.6	0.6	0.60	0.60	0.60	0.6	0.6	0.6
Vitamin and antibiotic mix[b]	1.0	1.0	1.0	1.00	1.00	1.00	1.0	1.0	1.0
Monosodium glutamate	2.2	4.2	6.2	0.03	2.03	4.03	--	--	--
L-lysine HCL	--	--	--	0.31	0.31	0.31	--	--	--
DL-methionine	--	--	--	0.27	0.27	0.27	--	--	--
Glycine	--	--	--	0.80	0.80	0.80	--	--	--
L-arginine HCL	--	--	--	0.14	0.14	0.14	--	--	--
DL-threonine	--	--	--	0.21	0.21	0.21	--	--	--
DL-valine	--	--	--	0.29	0.29	0.29	--	--	--
DL-histidine HCL	--	--	--	0.06	0.06	0.06	--	--	--
DL-tryptophan	--	--	--	0.05	0.05	0.05	--	--	--
DL-isoleucine	--	--	--	0.04	0.04	0.04	--	--	--

[a]Salt mix is shown in Appendix, Table 18.

[b]Vitamin and antibiotic mix is shown in Appendix, Table 17.

Results and discussion

Chick weight and feed efficiency data for experiment four are given in Table 9, and the analyses of variance of these data are given in Appendix, Table 24.

Increasing total crude protein from 18 to 22 percent by additional monosodium glutamate supplementation in the basal diets did not have any significant effect on chick growth at two or four weeks of age. At two weeks of age, analysis of variance indicated that there was a significant (P = .05 or less) improvement in feed efficiency as dietary protein was increased from 18 to 22 percent. However, this effect was not observed at the completion of the four week feeding period.

When the nine essential amino acids were supplemented to the basal diets described above, there were highly significant (P = .01 or less) improvements in chick weight gains and feed efficiency at both two and four weeks of age. These effects were anticipated, since similar observations had been made in preceding experiments with the 18 percent protein basal diet. It was of particular interest to note, however, that increasing total dietary protein concomittantly with essential amino acid supplementation had no significant effects on chick growth and feed efficiency. In addition, there was no apparent interaction between percent dietary protein and essential amino acid supplementation with respect to its effects on chick growth and feed efficiency. The fact

Table 9. Chick weight and feed efficiency data for experiment four

Dietary treatment	Chick weight (grams) Rep. 1	Rep. 2	Av.	Feed efficiency[a] Rep. 1	Rep. 2	Av.
2 weeks						
Basal						
18%	182.6[b]	200.4[b]	191.5	1.54	1.58	1.56
20%	187.2	193.8	190.5	1.57	1.53	1.55
22%	185.0	190.2	187.6	1.49	1.50	1.50
Basal + a.a.						
18%	229.9	205.5	217.7	1.42	1.43	1.42
20%	202.2	224.4	213.3	1.42	1.44	1.43
22%	225.6	209.8	217.7	1.37	1.42	1.40
Control						
18%	208.6	202.2	205.4	1.53	1.58	1.56
20%	223.6	225.7	224.6	1.40	1.40	1.40
22%	229.5	217.5	223.5	1.38	1.38	1.38

[a] Grams of feed per gram of gain.

[b] Average weight of 10 chicks per experimental pen.

Table 9. (Continued)

Dietary treatment	Chick weight (grams)			Feed efficiency[a]		
	Rep. 1	Rep. 2	Av.	Rep. 1	Rep. 2	Av.
<u>4 weeks</u>						
Basal						
18%	470.5[b]	433.7[b]	452.1	1.91	1.94	1.92
20%	473.7	474.0	473.8	1.82	1.92	1.87
22%	431.0	435.0	433.0	1.98	2.04	2.01
Basal + a.a.						
18%	556.0	508.1	532.0	1.63	1.71	1.67
20%	515.7	498.9	507.0	1.78	1.69	1.74
22%	561.0	507.2	534.1	1.56	1.82	1.69
Control						
18%	479.6	485.9	482.8	1.86	1.97	1.92
20%	536.3	539.1	537.[illegible]	1.61	1.69	1.65
22%	570.2	550.4	560.3	1.67	1.71	1.69

that this interaction was not noted indicates that the response in chick growth and feed efficiency due to amino acid supplementation of the 18 percent protein basal diet did not differ significantly from that observed when the same level of amino acids was supplemented to the basal diet containing 22 percent protein.

In contrast to that observed when dietary protein was increased in the basal diets, increasing dietary protein from 18 to 22 percent, in the control diets, by replacing corn with soybean oil meal did result in significant improvements in chick growth and feed efficiency. At two weeks of age, increasing dietary protein significantly (P = .01 or less) improved feed efficiency, even though the differences observed in chick growth were not statistically significant. At the end of four weeks, increasing dietary protein resulted in a significant (P = .05 or less) improvement in both chick growth and feed efficiency.

The data collected in this experiment strongly suggest that the dietary level of the essential amino acids has a greater influence on chick performance than does the dietary level of total crude protein. This observation becomes apparent when one compares the differences in chick performance due to increasing dietary protein between chicks fed the basal diets and those receiving the control diets. At each respective protein level, the only major difference between the basal and control diet was the amino acid composition, since both diets were formulated to be isocaloric and isoproteinous.

A secondary consideration of this experiment was to observe the influence of the various dietary treatments on serum cholesterol and serum protein concentrations. These concentrations are given in Tables 10 and 11, respectively, and the analysis of variance of the serum cholesterol data is given in the Appendix, Table 25.

Although it may appear that dietary treatment did have some influence on serum cholesterol, this observation could

Table 10. Blood serum cholesterol concentrations as affected by dietary treatment in experiment four

Dietary treatment	Serum cholesterol concentrations (mg./100 ml. serum)				
	Rep. 1[a]		Rep. 2[a]		Av.
Basal					
18%	190	170	170	175	177
20%	170	174	181	177	176
22%	195	202	191	176	191
Basal + a.a.					
18%	177	172	186	190	181
20%	136	129	178	194	159
22%	186	175	158	165	171
Control					
18%	156	140	188	191	169
20%	154	166	175	175	167
22%	166	167	157	137	156

[a]Values given represent duplicate determinations of a single pooled serum sample for each experimental pen.

Table 11. Blood serum protein concentrations as affected by dietary treatment in experiment four

Dietary treatment	Total serum protein	Albumin	Globulin
	Grams/100 ml. serum		
Basal			
18%	3.4[a]	2.4[a]	1.1[a]
20%	3.4	2.2	1.2
22%	3.5	2.4	1.1
Basal + a.a.			
18%	3.5	2.4	1.2
20%	3.5	2.4	1.1
22%	3.5	2.3	1.2
Control			
18%	3.5	2.3	1.2
20%	3.5	2.3	1.2
22%	3.6	2.2	1.4

[a]Values reported represent duplicate determinations from replicate experimental pens.

not be substantiated to any acceptable degree of probability when the data was subjected to statistical analysis. In some instances, it was noted that the differences in serum cholesterol concentrations between replicate experimental pens were greater than that observed between experimental treatments. Analysis of variance did indicate, however, that serum cholesterol concentrations of chicks receiving the control diets tended to be lower than serum cholesterol concentrations of chicks receiving the basal and the amino acid-supplemented basal diets. This difference approached statistical significance at the 5 percent level of probability.

There was no indication that dietary treatment had any appreciable influence on serum protein concentrations. As indicated in Table 11, the values reported represent the average of duplicate determinations of replicate experimental pens. The raw data was not subjected to statistical analysis because it was obvious that the greatest source of variation consistently occurred, not only between duplicate determinations of the same serum sample, but also between replicate groups fed the same experimental diets.

Experiment Five

Purpose

Having observed no appreciable effect on chick performance due to increased total crude protein and/or amino acid supplementation other than the supplementation of lysine, methionine and glycine to the 18 percent protein basal diet, it was desirable to formulate a new basal diet in which cellulose and excess monosodium glutamate would be excluded. Therefore, this experiment was designed to observe differences, if any, as measured by chick performance, between the basal diet used in previous experiments and a newly-formulated 18 percent protein basal diet.

Procedure

The experiment consisted of four dietary treatments, each duplicated, giving a total of eight experimental pens. Eighty day-old chicks were individually weighed, grouped into lots of ten chicks and assigned a battery brooder in accordance with a randomized block design.

The composition of experimental rations is given in Table 12. In the following discussion, the basal diet used in previous experiments will be referred to as Basal 1, the newly-formulated diet as Basal 2. Due to the exclusion of cellulose in Basal 2, it was necessary to decrease the level of soybean oil in order to achieve a diet that was isocaloric with Basal 1.

The level of dietary amino acid supplementation in Basal 2 is lower than that in Basal 1, due to an increased level of corn and soybean oil meal in Basal 2. On a calculated basis, however, the dietary levels of lysine, methionine and glycine are constant between the two amino acid-supplemented diets. As was the case in preceding experiments, amino acid supplementation was accomplished at the expense of monosodium glutamate.

Results and discussion

Chick weight and feed efficiency data for this experiment are given in Table 13, and the analyses of variance of these

Table 12. Composition of diets used in experiment five

Ingredient	Basal 1	Basal 1 + a.a.	Basal 2	Basal 2 + a.a.
	Percentage composition			
Ground yellow corn	59.2	59.20	66.7	66.70
Soybean oil meal, 50% protein	21.0	21.00	21.4	21.40
Soybean oil	9.0	9.0	6.0	6.00
Cellulose, alphacel	4.0	4.00	--	--
Dicalcium phosphate	2.0	2.00	2.0	2.00
Ground oystershell	1.0	1.00	1.0	1.00
Salt mix[a]	0.6	0.60	0.6	0.60
Vitamin and antibiotic mix[b]	1.0	1.00	1.0	1.00
Monosodium glutamate	2.2	0.82	1.3	0.04
L-lysine HCL	--	0.31	--	0.26
DL-methionine	--	0.27	--	0.24
Glycine	--	0.80	--	0.76
Calculated analysis[c]				
Protein, %	18.0	18.00	18.0	18.00
Productive energy, Calories per pound	1105	1105	1107	1107

[a]Salt mix is shown in Appendix, Table 18.

[b]Vitamin and antibiotic mix is shown in Appendix, Table 17.

[c]Values used to arrive at this analysis are given in Appendix, Table 16.

data are given in Appendix, Table 26.

At two weeks of age, there were no significant differences in chick weight and feed efficiency among any of the dietary treatments. At four weeks, however, chicks fed the amino acid-supplemented diets demonstrated significantly

Table 13. Chick weight and feed efficiency data for experiment five

Dietary treatment	Chick weight (grams) Rep. 1	Rep. 2	Av.	Feed efficiency[a] Rep. 1	Rep. 2	Av.
<u>2 weeks</u>						
Basal 1	205.6[b]	185.2[b]	195.4	1.64	1.80	1.72
Basal 1 + a.a.	212.9	192.8	202.9	1.50	1.50	1.50
Basal 2	203.8	182.0	192.9	1.64	1.66	1.65
Basal 2 + a.a.	205.1	199.7	202.4	1.46	1.62	1.54
<u>4 weeks</u>						
Basal 1	506.5	468.9	487.7	1.95	2.01	1.98
Basal 1 + a.a.	528.1	498.2	513.2	1.79	1.72	1.76
Basal 2	506.8	493.5	495.2	1.91	1.88	1.90
Basal 2 + a.a.	523.8	520.4	522.1	1.71	1.81	1.76

[a]Grams of feed per gram of gain.

[b]Average weight of 10 chicks per experimental pen.

(P = .05 or less) greater body weights and superior feed efficiency ratios than did chicks fed the basal diets.

The primary purpose of this experiment was to observe if chicks receiving Basal 1 differed in performance from chicks receiving Basal 2. The results of this experiment gave no indication that these diets differed to any significant degree in influencing chick weights or feed efficiency. Furthermore, the response of chicks to amino acid supplementation when fed the respective basal diets was not appreciably different.

It was concluded, therefore, that although certain modifications had been made in the formulation of a new 18 percent protein basal diet, chick performance observed with this diet did not differ to any significant degree from that observed with the 18 percent protein basal diet used in preceding experiments.

Experiment Six

Purpose

The primary objectives of this experiment were to observe the influence of crystalline amino acid supplementation on chick growth, feed efficiency and serum cholesterol concentrations in the growing chick.

Procedure

The experiment consisted of eight dietary treatments, each duplicated, giving a total of sixteen experimental pens. One hundred and sixty day-old chicks were individually weighed, grouped into lots of ten chicks and assigned a battery brooder in accordance with a 2 x 2 x 2 completely randomized factorial design.

Procedures used for the collection of blood samples and subsequent analysis for serum cholesterol are outlined in the Experimental Procedure section.

The composition of the basal diet used to conduct this trial was the same as Basal 2 given in Table 12. The remaining seven experimental diets were obtained by supplementation with lysine, methionine and glycine singly and in all possible combinations.

Results and discussion

Chick weight and feed efficiency data are given in Table 14, and the analyses of variance of these data are given in Appendix, Table 27.

As was expected in view of previous data collected, amino acid supplementation of the basal diet resulted in improved chick weight gains. At two weeks of age, chicks whose diets had been supplemented with lysine, methionine and glycine in combination were 13 percent heavier than chicks fed the basal

Table 14. Chick weight and feed efficiency data for experiment six

Dietary treatment	Chick weight (grams) Rep. 1	Rep. 2	Av.	Feed efficiency[a] Rep. 1	Rep. 2	Av.
<u>2 weeks</u>						
Basal	177.9[b]	175.4[b]	176.7	1.51	1.46	1.48
+ lysine (L)	168.7	178.0	173.4	1.54	1.52	1.53
+ methionine (M)	185.1	177.2	181.2	1.48	1.56	1.52
+ glycine (G)	172.6	174.5	173.6	1.44	1.55	1.50
+ L + M	185.6	196.0	190.8	1.43	1.59	1.51
+ L + G	179.1	181.3	180.2	1.40	1.44	1.42
+ M + G	175.8	177.9	176.8	1.50	1.53	1.52
+ L + M + G	193.0	206.7	199.8	1.36	1.39	1.37
<u>4 weeks</u>						
Basal	468.6	453.2	460.9	1.94	1.92	1.93
+ L	467.1	457.0	462.0	1.99	1.76	1.88
+ M	479.7	489.3	484.5	1.84	1.91	1.88
+ G	445.9	457.2	451.6	1.92	1.86	1.89
+ L + M	522.4	513.5	517.9	1.66	1.80	1.73
+ L + G	475.1	491.1	483.6	1.83	1.75	1.79
+ M + G	486.2	481.3	483.8	1.73	1.78	1.76
+ L + M + G	529.0	511.4	520.0	1.69	1.65	1.67

[a]Grams of feed per gram of gain.

[b]Average of 10 chicks per experimental pen.

diet. The majority of the improvement in body weight could be attributed to lysine and methionine supplementation, since chicks fed a diet containing only these two supplemental amino acids were 9 percent heavier than chicks fed the basal diet.

Analysis of variance of the data revealed that there were highly significant (P = .01 or less) main effects on chick growth due to lysine and methionine supplementation, in addition to a statistically significant (P = .05 or less) lysine x methionine interaction. This interaction indicates that the response in growth to single additions of either lysine or methionine differed from that observed when both amino acids were supplemented in combination.

At four weeks of age, approximately 97 percent of the improved body weight gains, due to supplemental amino acids, could be accounted for by lysine and methionine supplementation. Chicks fed the diet supplemented with all three amino acids were 12.8 percent heavier than chicks fed the basal diet, whereas chicks receiving the diet supplemented with lysine and methionine in combination were 12.4 percent heavier than chicks receiving the basal diet.

Upon statistical analysis, highly significant (P = .01 or less) main effects for lysine and methionine supplementation were revealed. In contrast to that observed at two weeks, no significant interactions were found to be present. This analysis might lead one to conclude that supplementation of the basal diet with lysine or methionine alone would result in a significant improvement in chick weight. From the data presented in Table 14, however, it is obvious that supplementation with lysine alone did not improve chick performance. Although supplementation of the basal diet with methionine alone did improve weight gains by 4.5 percent, the full benefit of amino acid supplementation did not occur until both lysine and methionine were supplemented in combination.

Even though chicks fed the diet supplemented with all three amino acids showed an 8 percent improvement in feed efficiency over that observed for chicks fed the basal diet at two weeks of age, no statistically significant differences in feed efficiency were found among any of the dietary treatments. At four weeks of chick age, however, a significant ($P = .05$ or less) improvement in feed efficiency was observed when the basal diet was supplemented with lysine and/or methionine. As discussed earlier, it is obvious that the full benefit of supplementation with these two amino acids did not occur unless lysine and methionine were supplemented in combination, even though statistical analysis did not indicate a significant interaction between these two amino acids.

In experiment two, it was concluded that a maximal response in chick performance could not be achieved unless the basal diet was supplemented with lysine, methionine and glycine in combination. Although the basal diets used in experiments two and six were different, the amino acid composition of these basal diets, due to changes in formulation, was not appreciably altered, as indicated in Table 12. Furthermore, in each experiment, the dietary level of lysine, methionine and glycine was identical on a calculated basis. Yet in the former experiment, a significant response to glycine supplementation was observed, whereas in the latter experiment, no significant improvement in chick performance could be attributed to glycine supplementation.

The contrast in results between these two experiments may raise a serious question as to the validity of the conclusions made in experiment two. It will be recalled that in experiment two a significant lysine x methionine x glycine interaction was found to be present only at two weeks, while at four weeks this interaction was not significant at P = .05. However, there was a strong indication that supplemental glycine improved chick performance in the presence of supplemental lysine, and lysine supplementation was only effective in the presence of supplemental methionine. In the presently reported experiment, it appeared that supplemental glycine did have some beneficial effect on chick performance up to two weeks of age, but not at the end of four weeks of chick age. However, a maximal response in chick weight and feed efficiency occurred only when glycine was supplemented in the presence of lysine and methionine in both experiments two and six, even though, statistically, this observation could not be fully substantiated in the latter experiment.

The second objective of this experiment was to observe the influence of specific amino acid supplementation on serum cholesterol concentrations. These data are given in Table 15, and the analysis of variance of the data is given in Appendix, Table 28.

Chicks fed diets supplemented with lysine had significantly (P = .01 or less) lower serum cholesterol concentrations than did chicks receiving diets without supplemental

Table 15. Blood serum cholesterol concentrations as affected by dietary treatment in experiment six

Dietary treatment	Serum cholesterol concentration (mg./100 ml. serum)				
	Rep. 1[a]		Rep. 2[a]		Av.
Basal	188	180	151	163	170
+ lysine (L)	144	147	131	137	140
+ methionine (M)	166	166	151	153	159
+ glycine (G)	160	166	174	170	168
+ L + M	124	126	145	151	136
+ L + G	160	161	143	133	149
+ M + G	204	222	145	152	181
+ L + M + G	172	163	180	191	176

[a]Values given represent duplicate determinations of a single pooled serum sample for each experimental pen.

lysine. In contrast, chicks receiving supplemental glycine demonstrated significantly (P = .05 or less) higher serum cholesterol concentrations when compared to chicks not receiving supplemental glycine. The response in serum cholesterol concentrations due to supplemental methionine in the presence of supplemental glycine was found to be significantly different (P = .05 or less) from that observed when glycine was not supplemented. Methionine, when added in the absence of supplemental glycine, resulted in slightly lower serum cholesterol concentrations, whereas, higher serum cholesterol concentrations were observed when methionine was added in the presence of supplemental glycine.

The nature of the results found in this part of the investigation are difficult to explain when compared with growth rate data. From this experiment, there appears to be no correlation between growth rate and serum cholesterol concentrations as the amino acid balance of the basal diet was improved by amino acid supplementation.

DISCUSSION AND SUMMARY

Amino Acid Supplementation of a Corn-Soybean Oil Meal Chick Diet

The first three experiments conducted for this dissertation demonstrate that the 18 percent protein basal diet was deficient in certain essential amino acids necessary to achieve a maximal growth rate and feed efficiency. By supplementing the basal diet with crystalline amino acids up to the levels reported by Dean and Scott (1962), it was found that the inclusion of only lysine, methionine and glycine was necessary in order to achieve a proper amino acid balance which resulted in maximal growth rate. Compared to the 18 percent protein standard diet reported by these workers, the basal diet formulated for the presently reported experiments was also deficient in six other amino acids. However, it was found that the addition of these six amino acids did not significantly improve chick growth and feed efficiency over that observed when only lysine, methionine and glycine were supplemented. In contrast, it was observed that if threonine was supplemented alone, an amino acid imbalance occurred which tended to depress chick growth. This imbalance was corrected in part or in whole by the supplementation of valine in combination with threonine.

It is reasonable to expect that observations concerning the chicks' requirement for a given essential amino acid would differ depending upon the dietary source of that amino acid. In the standard diet reported by Dean and Scott (1962) all the amino acids were supplied in the free, crystalline form, whereas, the majority of the amino acids supplied in the presently reported experiments were from intact protein sources. Furthermore, the isomeric form(s) of the crystalline amino acids supplemented, either D, L or DL, used in any particular experiment would also be expected to influence the apparent requirement for any given amino acid.

When an intact protein is hydrolyzed in the gastrointestinal tract, only the L isomer of the amino acids is obtained. Enzymatic hydrolysis of tissue proteins _in vitro_ likewise yields only the L isomer of the amino acids. These observations strongly support the view that the D isomer of amino acids is not utilized directly in the synthesis of tissue proteins. The observation that the D isomer of an essential amino acid, with the exception of methionine, is not utilized by the chick as efficiently as is the L isomer has been reported among others by Fell _et al._ (1959).

Dean and Scott (1962) used only the L isomer of the amino acids, with the exception of methionine, in developing their amino acid standard diet. In the present experiments, the DL racemic form of crystalline amino acids, with the exception of lysine and arginine, was used for supplementation. Because of differences in utilization of the isomeric form(s) of amino acids, it would be logical to assume that the dietary requirement for a given amino acid supplied only as the L isomer would be less than that observed when part of the total amino acid was supplied as the racemic mixture. It appears, however, from the experiments reported here, with a corn-soybean oil meal diet, that the chicks' amino acid requirement for arginine, threonine, valine, histidine, tryptophan and isoleucine is lower than that reported by the Illinois workers. These results are in contrast to the hypothesis stated above.

There are two factors, however, which must be considered as possible explanations as to why Dean and Scott (1962) observed a higher requirement for these amino acids than was observed in these experiments. First, we are still faced with the problem of estimating the amino acid composition of intact proteins from values that were determined by microbiological assay a number of years ago. In view of newer and more reliable assay methods, the values used to obtain the amino acid composition of corn and soybean oil meal in the basal diet may or may not reflect the true amino acid composition of these protein sources.

Secondly, differences in availability of amino acids for absorption from the gastrointestinal tract between a purified diet and a diet which contains intact proteins may also affect the apparent requirement for a given amino acid. In the former diet, the amino acids are readily available for absorption without aid of certain enzymatic digestive processes. In contrast, a diet containing intact proteins must be subjected to a selective degradative process, which results in the release of individual amino acids in a relatively slow and orderly fashion. For a period of time immediately following ingestion of a purified diet, it would be anticipated that there would be an excess of amino acids present in the gastrointestinal tract. This excess of amino acids would probably result in a lower efficiency of absorption than that observed when an intact protein is fed. If there was an excessive quantity of amino acids in the gastrointestinal tract, a high blood plasma amino acid concentration would also be expected. Thus, when a purified diet is fed, the concentration of a given amino acid in the blood plasma may be greater than the body tissues' ability to efficiently utilize that amino acid. This would result in a further loss of the amino acid through deamination and/or excretion in the urine.

In addition to other factors--such as energy, vitamin and mineral interrelationships--the above mentioned factors must be viewed individually and in combination when attempting to explain differences in apparent amino acid requirements as reported by various experiment stations.

The concept that tissue protein synthesis, assuming that weight gain is a valid index of this synthesis, can proceed only up to a point where one amino acid becomes the most limiting, regardless of the adequacy or inadequacy of any other essential amino acid, was illustrated in experiment two. In this experiment, the supplementation of lysine and glycine individually did not result in any apparent improvement in chick growth and feed efficiency, whereas, the supplementation of methionine individually did appear to result in a slight improvement in growth rate. However, when lysine was supplemented in the presence of methionine, a further improvement in growth rate was observed. Furthermore, a response from glycine supplementation was not observed unless both lysine and methionine had been supplemented to the basal diet. Even though the basal diet was deficient in all three amino acids, the supplementation of any one amino acid did not result in a growth response unless the needs for the most limiting amino acid in the diet had first been met.

The merit of designing an experimental diet in which the essential amino acid composition and total crude protein can be altered without modification of the levels of intact protein sources is shown in experiment four.

The three diets called "control diets" in this experiment were formulated by altering the corn-soybean oil meal ratio to result in diets of 18, 20 and 22 percent protein. As the percent protein of the diets was increased, an improved growth rate of chicks was observed. In contrast, when experimental chicks were fed the "basal diets", in which dietary protein was increased by monosodium glutamate, no improvement in growth rate was observed.

If one were to restrict his observation to the results obtained with the control diets, it could be validly questioned as to whether the growth response was due to an increased total nitrogen intake or to a more favorable amino acid balance. It has been observed in this labratory that the 22 percent protein control diet is deficient in methionine. Many experimenters feel that the amino acid requirement should be expressed as a percentage of total dietary protein and not as percentage of total diet. If this criterion was accepted, the 22 percent protein control diet would be considered more deficient in methionine than the 18 percent control diet. Thus, these experimenters might argue that the growth response observed, as the dietary protein of the control diets was increased, is due to an increased nitrogen intake and not due to an improvement in the amino acid balance of the diet.

It has already been pointed out that, as dietary protein was increased without altering the essential amino acid composition in the basal diets, no improvement in growth rate was observed. Chick growth was improved, however, when the limiting essential amino acids had been supplemented to these diets. At the same time, no further improvement in chick growth was observed when total crude protein was increased, even after essential amino acid supplementation had been accomplished.

These results lead this investigator to conclude that the essential amino acid requirements should be expressed as percent of total diet and not as percent of dietary protein. Furthermore, it is concluded that the dietary level of a given essential amino acid has a greater influence on growth rate than does total nitrogen intake, in diets of the nature as formulated to conduct these studies.

These conclusions are based, however, on the assumption that monosodium glutamate is a readily available and an efficiently utilized nitrogen source for the chick. Although every effort was made to hold all dietary nutrients, other than essential amino acids and total crude protein, constant in the formulation of the experimental diets in these studies, it must be pointed out that the use of monosodium glutamate to increase total crude protein also increases the sodium intake. Whether or not the level of dietary sodium reached a point to result in a toxicity or any other adverse effect on growth must be further investigated. However, from the observations made in the presently reported experiments, there was no direct evidence to indicate that the use of monosodium glutamate as a nitrogen source resulted in any adverse influence on chick growth and/or feed efficiency.

As indicated in the Introduction, the primary objective of these experiments was to investigate the feasibility of obtaining a rapid and efficient chick growth by specific amino acid supplementation to an 18 percent protein diet. The protein level selected to conduct these studies is lower than that normally recommended to obtain maximal chick growth rates. Although it was not possible to obtain as good a growth rate with this diet as observed with the 22 percent protein control diet, it is felt that sufficient progress was made to indicate that this goal may be reached through further experimentation. Even though it is known that the 22 percent protein control diet still will not support a maximal chick growth rate, due to a methionine deficiency and absence of unidentified growth factors, there are several areas of experimentation yet to be accomplished with the 18 percent protein diet formulated for these experiments. For example, was phenylalanine a limiting amino acid in the basal diet? According to the National Research Council's recommendations, the basal diet is deficient in this essential amino acid, whereas, it is adequate according to the standard diet reported by Dean and Scott (1962). Secondly, was the chicks' true dietary requirement for any given essential amino acid fully met in these studies? Experiments designed to investigate the effect of various levels of essential amino acid supplementation to the basal diet may answer this question.

Effect of Dietary Protein on Serum Protein Concentrations

There was no evidence found in experiment four to indicate that the level of dietary protein and/or amino acid balance had any significant effect on serum protein concentrations. This is in contrast to observations of other workers as reported in the Review of Literature section.

There are two factors, however, that may have lead to the differing results obtained, and the conclusions made, in the presently reported experiments. First, if any of the dietary treatments imposed upon the experimental chicks did have a small, but still significant, influence on serum proteins, it is thought that the procedure used to make these physiological measurements was not reliable enough to detect small differences. This statement is based upon the fact that an appreciable variation was encountered between duplicate determinations of a single pooled serum sample, indicating that either the procedure employed and/or the experimenter's techniques were not refined enough to detect small changes in serum protein concentrations as affected by dietary treatment.

Secondly, in the majority of experiments reported in the literature, the levels of dietary protein ranged from a suboptimal level (10 percent or less) to a super-normal level (30 percent or greater). In general, a significant difference in serum protein concentrations was observed only when comparing those values of chicks fed the sub-optimal dietary protein level with those values of chicks receiving a normal or super-normal dietary protein level. In experiment four, the dietary protein ranged from 18 to 22 percent, neither of which could be considered as sub-optimal or super-normal when compared to those levels used in previously reported experiments. Thus, the dietary treatments imposed in experiment four may not have been severe enough to result in any significant alteration in the serum protein concentrations, even though growth rate and feed efficiency were significantly affected by these dietary treatments.

Effect of Dietary Protein on Serum Cholesterol Concentrations

Similar to observations made concerning serum protein concentrations, it is thought that little, if any, meaningful data was obtained to conclusively demonstrate that dietary protein level and/or amino acid balance had any significant effect on chick serum cholesterol concentrations.

The majority of experimental evidence cited in the Review of Literature section tends to support the hypothesis that serum cholesterol concentrations are decreased as the protein nutrition of the chick is improved. In certain instances, it has been reported that specific amino acid supplementation has resulted in significantly higher or lower serum cholesterol concentrations, which in turn were unrelated to growth rate. This latter observation is similar to that noted in experiment six, where it was found that lysine supplementation resulted in lower serum cholesterol concentrations, whereas, glycine supplementation increased serum cholesterol concentrations.

If serum cholesterol concentrations could be related to growth rate, which in turn is influenced by protein nutrition, a feasible hypothesis to explain the correlation between these observations might be advanced. As is the case in many studies, however, there seldom seems to be enough experimental evidence available to scientifically explain every biological variation within a given experiment. The experiments conducted for this dissertation concerning the effects of dietary protein on serum cholesterol concentrations were not an exception.

CONCLUSIONS

1. When supplementing the 18 percent protein basal diet formulated for these experiments with crystalline amino acids, up to levels reported by Dean and Scott (1962), only lysine, methionine and glycine supplementation is necessary to achieve maximal chick growth and feed efficiency.

2. In the basal diet, methionine is the first limiting amino acid, followed by lysine, then glycine. Although all three of these amino acids are deficient in the basal diet, a significant response in chick weight gains is not observed unless the most limiting amino acid has been first supplemented.

3. A maximal response in weight gains and feed efficiency is not observed unless the basal diet is supplemented with lysine, methionine and glycine in combination. Further supplementation of the basal diet with arginine, threonine, valine, histidine, tryptophan and isoleucine to the dietary levels selected does not improve chick growth and feed utilization.

4. Increasing dietary protein from 18 to 22 percent with monosodium glutamate in the basal and/or amino acid-supplemented basal diets does not result in any significant improvement in chick weight gains. However, increasing dietary protein from 18 to 22 percent, by substituting soybean oil meal for corn, results in an improved chick weight and feed effi-

ciency.

5. The level of dietary protein and/or amino acid balance had no meaningful effects on serum protein and serum cholesterol concentrations.

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APPENDIX

Table 16. Composition of corn, soybean oil meal and soybean oil used in diet formulation

	Corn	Soybean oil meal	Soybean oil
Protein	9.00[a]	50.00[a]	--
Productive energy	1145.00	790.00	2900.00
Lysine	0.30	3.00	--
Methionine	0.18	0.87	--
Cystine	0.16	0.68	--
Glycine	0.40	2.70	--
Arginine	0.50	3.10	--
Threonine	0.40	1.94	--
Valine	0.41	2.43	--
Histidine	0.32	1.19	--
Isoleucine	0.35	2.64	--
Tryptophan	0.10	0.58	--
Leucine	1.10	3.74	--
Phenylalanine	0.45	2.47	--

[a]All values reported are expressed as percent of total with the exception of productive energy, which is expressed as Calories per pound.

Table 17. Vitamin and antibiotic mix

Constituent	Mix added the following per pound of ration
Vitamin A	3,000.0 I.U.
Vitamin D_3	500.0 I.C.U.
Vitamin E	5.0 I.U.
Menadione	1.0 mg.
Riboflavin	4.0 mg.
Niacin	24.0 mg.
Calcium pantothenate	4.0 mg.
Choline	300.0 mg.
Vitamin B_{12}	7.5 mcg.
Penicillin	5.0 mg.

Table 18. Salt mix

Constituent	Mix added the following per pound of ration
Sodium chloride	2.3 gm.
Manganese	34.2 mg.
Zinc	13.8 mg.
Iron	13.8 mg.
Copper	1.4 mg.

Table 19. Analyses of variance of chick weight and feed efficiency data for experiment one

Source of variation	d.f.		Chick weight m.s.	Feed efficiency m.s.
2 weeks				
Treatment		2	566.56**	0.050**
Protein	1		852.85**	0.060**
Amino acid	1		294.29*	0.040**
Error		6	32.97	0.002
		8		
4 weeks				
Treatment		2	5,508.71**	0.040**
Protein	1		8,637.37**	0.020*
Amino acid	1		2,380.04**	0.060**
Error		6	119.64	0.002
		8		

*Significant at P = .05 or less.

**Significant at P = .01 or less.

Table 20. Analyses of variance of chick weight and feed efficiency data for trial one of experiment two

Source of variation	d.f.		Chick weight m.s.	Feed efficiency m.s.
<u>2 weeks</u>				
Treatment		7	94.99	0.007
Lysine (L)	1		163.21	0.022
Methionine (M)	1		43.90	0.002
Glycine (G)	1		28.36	0.001
L + M	1		70.13	0.002
L + G	1		122.65	0.002
M + G	1		0.27	0.020
L + M + G	1		236.40*	0.003
Error		8	48.80	0.008
		15		
<u>4 weeks</u>				
Treatment		7	540.01*	0.014*
L	1		1,004.89*	0.028*
M	1		772.84*	0.006*
G	1		158.76	0.001
L + M	1		707.56*	0.140*
L + G	1		615.04*	0.001
M + G	1		10.24	0.014*
L + M + G	1		510.76	0.031*
Error		8	112.36	0.003
		15		

*Significant at P = .05 or less.

Table 21. Analyses of variance of chick weight and feed efficiency data for trial two of experiment two

Source of variation	d.f.		Chick weight m.s.	Feed efficiency m.s.
2 weeks				
Treatment		7	50.93	0.006
Arginine (A)	1		13.69	0.009
Threonine (T)	1		15.60	0.003
Valine (V)	1		60.06	0.001
A + T	1		6.77	0.011
A + V	1		108.17	0.005
T + V	1		60.07	0.004
A + T + V	1		189.44	0.007
Error		8	105.58	0.010
		15		
4 weeks				
Treatment		7	364.73	0.004
A	1		224.25	0.003
T	1		556.85	0.004
V	1		237.93	0.005
A + T	1		191.92	0.002
A + V	1		41.92	0.002
T + V	1		1,253.25*	0.001
A + T + V	1		47.01	0.013
Error		8	188.50	0.003
		15		

*Significant at P = .05 or less.

Table 22. Analyses of variance of chick weight and feed efficiency data for trial three of experiment two

Source of variation	d.f.	Chick weight m.s.	Feed efficiency m.s.
<u>2 weeks</u>			
Treatments	7	39.22	0.002
Histidine (H)	1	48.65	0.001
Tryptophan (T)	1	61.23	0.002
Isoleucine (I)	1	38.75	0.002
H + T	1	6.63	0.001
H + I	1	45.23	0.001
T + I	1	21.86	0.003
H + T + I	1	52.19	0.004
Error	8	26.16	0.001
	15		
<u>4 weeks</u>			
Treatments	7	210.89	0.005
H	1	22.09	0.006
T	1	852.64	0.013*
I	1	1.69	0.007
H + T	1	499.52	0.006
H + I	1	4.20	0.002
T + I	1	81.62	0.002
H + T + I	1	30.26	0.001
Error	8	409.06	0.002
	15		

*Significant at P = .05 or less.

Table 23. Analyses of variance of chick weight and feed efficiency for experiment three

Source of variation	d.f.	Chick weight m.s.	Feed efficiency m.s.
2 weeks			
Blocks	2	75.06	0.001
Treatments	2	618.32*	0.043**
Amino acids	1	1,192.34**	0.084**
3 vs. 9	1	44.29	0.001
Error	4	49.19	0.002
	8		
4 weeks			
Blocks	2	6.13	0.004
Treatments	2	4,483.29*	0.089**
Amino acids	1	8,951.22**	0.140**
3 vs. 9	1	15.36	0.007
Error	4	363.99	0.006
	8		

*Significant at P = .05 or less.

**Significant at P = .01 or less.

Table 24. Analyses of variance of chick weight and feed efficiency data for experiment four

Source of variation	d.f.		Chick weight m.s.	Feed efficiency m.s.
2 weeks				
Treatment		8	433.79*	0.0111**
Control vs. others	1		876.16*	0.0032*
Protein/control	2		233.16	0.0183**
Protein/basals	2		7.77	0.0028*
A.A./basals	1		2,085.60**	0.0420**
P. x A.A./basals	2		13.35	0.0006
Error		9	106.37	0.0005
		17		
4 weeks				
Treatment		8	3,760.85**	0.0364
Control vs. others	1		5,844.60**	0.0169
Protein/control	2		3,197.23*	0.0408*
Protein/basals	2		81.95	0.0034
A.A./basals	1		15,317.69**	0.1680**
P. x A.A./basals	2		1,183.08	0.0089
Error		9	405.45	0.0093
		17		

*Significant at P = .05 or less.

**Significant at P = .01 or less.

Table 25. Analysis of variance of serum cholesterol concentrations for experiment four

Source of variation	d.f.	Cholesterol m.s.
Treatment	8	455
Control vs. others	1	1,089
Protein/control	2	176
Protein/basals	2	428
A.A./basals	1	323
P. x A.A./basals	2	513
Error	27	266
	35	

Table 26. Analyses of variance of chick weight and feed efficiency data for experiment five

Source of variation	d.f.	Chick weight m.s.	Feed efficiency m.s.
<u>2 weeks</u>			
Blocks	1	572.91*	0.004
Treatments	3	50.03	0.029
Basals	1	4.35	0.001
Amino acids	1	143.65	0.054
B. x A.A.	1	2.10	0.006
Error	3	29.79	0.0[illegible]7
	7		
<u>4 weeks</u>			
Blocks	1	856.21	0.001
Treatments	3	452.83	0.024
Basals	1	229.98	0.003
Amino acids	1	1,123.39*	0.065*
B. x A.A.	1	5.13	0.004
Error	3	108.10	0.003
	7		

*Significant at P = .05 or less.

Table 27. Analyses of variance of chick weight and feed efficiency data for experiment six

Source of variation	d.f.		Chick weight m.s.	Feed efficiency m.s.
<u>2 weeks</u>				
Treatment		7	171.36*	0.0[illegible]63
Lysine (L)	1		332.60**	0.0086
Methionine (M)	1		504.00**	0.0001
Glycine (G)	1		18.06	0.0150
L + M	1		206.05*	0.0038
L + G	1		127.12	0.0162
M + G	1		0.26	0.0005
L + M + G	1		11.43	0.0002
Error		8	28.99	0.0031
		15		
<u>4 weeks</u>				
Treatment		7	1,292.74**	0.0167
L	1		2,631.69**	0.0317*
M	1		5,535.36**	0.0518*
G	1		43.56	0.0233
L + M	1		345.96	0.0013
L + G	1		278.89	0.0001
M + G	1		26.01	0.0007
L + M + G	1		187.69	0.0028
Error		8	78.75	0.0057
		15		

*Significant at P = .05 or less.

**Significant at P = .01 or less.

Table 28. Analysis of variance of serum cholesterol concentrations for experiment six

Source of variation	d.f.	Cholesterol m.s.
Treatment	7	1,113*
Lysine (L)	1	2,869**
Methionine (M)	1	332
Glycine (G)	1	2,329*
L + M	1	247
L + G	1	477
M + G	1	1,526*
L + M + G	1	13
Error	24	299
	31	

*Significant at P = .05 or less.